A Comparison of the Efficacy of a Live and Four Inactivated Vaccine Preparations for the Protection of Cats Against Experimental Challenge with Chlamydia psittaci

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ABSTRACT

A commercial live feline-chlamydial vaccine and four experimental inactivated preparations were compared on the basis of clinical protection in cats challenged conjunctivally and intranasally with Chlamydia psittaci. Best protection was afforded by the live vaccine. Good results were also obtained using inactivated preparations of a recent feline conjunctival isolate. Protection did not correlate with the development of complement fixing antibodies but may be related to the induction of a cell mediated response as assessed by the lymphocyte blastogenesis test.

RÉSUMÉ

Cette étude consistait à comparer l'efficacité d'un vaccin commercial vivant et de quatre préparations expérimentales inactivées, à l'endroit de Chlamydia psittaci, en se basant sur la protection clinique qu'ils conféraient à des chats soumis à une infection de défi, avec cette chlamydie, par les voies conjonctivale et intra-nasale. Le vaccin vivant conféra la meilleure protection. Les préparations inactivées qui provenaient d'un isolat récent d'un cas clinique de conjonctivite féline, donnèrent aussi de bons résultats. La protection ne concorda pas avec le développement d'anticorps fixateurs du complément; elle pourrait cependant être reliée à l'induction d'une immunité cellulaire, comme le pensèrent les auteurs, à la suite de l'épreuve de la blastogénèse des lymphocytes.

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INTRODUCTION

Chlamydiae have been recognized as the cause of a variety of naturally occurring diseases in many species of animals and birds (21, 24). In cats, *Chlamydia psittaci* infection was originally considered responsible for outbreaks of severe upper respiratory disease (2). As other organisms, particularly feline rhinotracheitis and calici viruses, became established as more frequently occurring pathogens many investigators began to doubt the significance of chlamydiae. Nevertheless, scattered reports of conjunctivitis (7, 19, 20, 23, 29) and rarely, generalized infection (4) continue to appear and the prophylactic use of commercial vaccines continues to be investigated (3, 8, 13, 17).

Most *C. psittaci* vaccine studies in cats have utilized live chlamydial yolk sac suspensions. Investigations using one commercial vaccine of this type have produced results varying from near complete protection (3, 6) to none at all (8, 22). Experimental trials with a different commercial preparation claim significant reduction in the severity and duration of clinical signs after vaccination (13, 17).

There is only one reported feline study where the efficacy of killed preparations

TABLE I. Composition of the *C. psittaci* Vaccine Preparation Used For Each Group of Cats

Group	Number of Cats	Vaccine	Adjuvant	Titer (ELD_{50}/mL)[a]
1	6	not vaccinated		
2	6	Strain A, live[b]	none	$10^{4.3}$
3	6	Strain A, inactivated	L75[c]	$10^{7.3}$
4	6	Strain B, inactivated	none	$10^{7.5}$
5	6	Strain B, inactivated	L75	$10^{7.5}$
6	6	Strain B, inactivated	FD19[d]	$10^{7.4}$

[a]Titer estimated from the titer of suspension prior to inactivation and adjusted for dilution with adjuvant
[b]Feline Pneumonitis Vaccine, Fromm Laboratories, Grafton, Wisconsin
[c]L75 adjuvant. Langford Laboratories, Guelph, Ontario. An adsorbed bacterial cell origin adjuvant, added to the chlamydial suspension in a ratio of 3 parts suspension to 1 part adjuvant
[d]FD19 adjuvant. Fort Dodge Laboratories, Fort Dodge, Iowa. An oil adjuvant, added to the chlamydial suspension in a ratio of 5 parts suspension to 1 part adjuvant

was evaluated (16). This study employed irradiated or crystal violet treated, purified yolk sac suspensions. These induced protection comparable to that of live organisms similarly purified but inferior to a live crude yolk sac preparation.

Experimental trials using live *C. trachomatis* vaccines in baboons have provided evidence for the multiplication of vaccine organisms in the spleen, lymph nodes and local tissues of inoculated animals (10, 18). Although similar investigations have not been reported in cats the potential disease hazard implicit in the baboon studies emphasizes the desirability of an inactivated vaccine.

The following experiment was designed to evaluate some alternative inactivated *C. psittaci* vaccine preparations in cats.

MATERIALS AND METHODS

VACCINE PREPARATION

Two strains of *C. psittaci* were used.

Strain A: yolk sac propagated from commercial live vaccine[1]. Infected yolk sacs of the second passage from the vaccine were prepared as a crude 20% suspension in phosphate buffered saline[2].

Strain B: the fifth passage yolk sac harvest of an isolate from a clinical case of feline conjunctivitis was prepared as strain A.

Titrations of the ELD_{50} (50% embryo lethal dose) of each suspension were performed by yolk sac inoculation of seven day old embryonated hens' eggs. Preparations were confirmed free of viruses, mycoplasmas or bacteria by conventional culture methods.

Low level thermal inactivation, 45°C for seven days, was chosen as a method which would inactivate the chlamydiae without major alteration in the antigenic structure of the organisms (26). Aliquots were verified inactivated by four passages in embryonated eggs.

Four alternative inactivated suspensions using different strains, with or without adjuvants, were evaluated and compared to the commercial live vaccines. These prepaarations are outlined in Table I.

CATS

Thirty-six specific pathogen free cats (5) 16 to 20 weeks old, of both sexes, from a colony maintained at the University of Guelph were randomly divided into six groups of six cats each as indicated in Table I.

VACCINATION PROTOCOL

Cats in groups 2 to 6 each received 0.1 mL of the appropriate vaccine, intramuscularly, at the initiation of the trial. The inoculation was repeated after three weeks.

CHALLENGE

Twenty-one days after the last vaccination, all cats were challenged with 0.5 mL

[1]**Feline Pneumonitis Vaccine, Fromm Laboratories Inc., Grafton, Wisconsin. (lot no. 35238).**

[2]**#310-4080 Gibco, Grand Island, New York.**

TABLE II. Protocol for Scoring Clinical Signs in Cats Following Chlamydial Challenge

Sign	Score
Conjunctivitis	
ocular discharge — slight	1
moderate	2
heavy	3
conjunctival hyperemia	1
chemosis	1
Nasal discharge — slight	1
heavy	2
Sneezing/coughing	1

Each eye was scored separately, the final score included the sum for both eyes. The maximum daily score possible for one eye was 5, for one cat 13.

of a 10^4 ELD_{50}/mL suspension of virulent Cello Strain *C. psittaci* (6) by droplet instillation onto the conjunctivae and intranasally.

OBSERVATIONS AND SAMPLE COLLECTION

All cats were observed daily for clinical disease starting one week prior to challenge and ending 22 days postchallenge. Signs were scored according to the protocol outlined in Table II.

Conjunctival swabs for chlamydial isolation were obtained prior to challenge and seven days after challenge. Swabs for viral, mycoplasmal and bacterial isolation were taken at the same time.

Sera for complement fixation tests were obtained by jugular puncture prior to vaccination, before challenge and three weeks postchallenge and stored at —20°C. Prior to test 0.5 mL of each sample was absorbed overnight at 4°C with 1 mg sterile yolk sac powder, clarified by centrifugation at 500 g for 10 min and inactivated at 56°C for 30 min. Group specific ornithosis (*C. psittaci*) antigen and uninfected yolk sac control antigen[3] were used throughout. Tests were performed in 96 well polystyrene microtiter plates[4] using 25 µL volumes as outlined previously (11).

Whole blood for the lymphocyte blastogenesis test was collected after jugular puncture into 5 mL vacutainers[5] containing 143 USP units of sodium heparin for each mL of blood. Stimulated cultures were prepared by combining 0.8 mL RPMI 1640[6] (supplemented with 20% fetal calf serum[7], 100 mg/mL streptomycin[8], 100 mg/mL vancomycin[9] and 10 mg/mL gentamicin[10]) 0.1 mL of mitogen or antigen and 0.1 mL of blood. An unstimulated control culture and a mitogen stimulated control were included for each blood sample tested. The latter contained 25 mg of concanavalin A[11], shown in a preliminary trial to be the optimal stimulating dose for feline whole blood incubated under the following conditions. *Chlamydia psittaci* antigens consisted of yolk sac suspensions diluted in RPMI 1640 to yield a titer of 10^5 ELD_{50} per culture. Two hundred µL aliquots of each suspension were pipetted into adjacent wells of a flat-bottomed microtiter plate[12] and incubated at 37°C in 5% CO_2 for 48 hours. Cultures were then pulsed with 0.5 µCi of tritiated thymidine[13] in 20 µL RPMI 1640 per well and incubated for an additional 18 hours. Samples were harvested using a Titerek Cell Harvester[14] onto glass filter papers[14], dried at 37°C for one hour, then transferred to scintillation vials[15]. Five mL of a scintillation cocktail (100 mg POPOP[16], 5.0 g POP[17]

[3]Ornithosis Antigen and Ornithosis Control Antigen, Canadian Hoescht, Montreal, Quebec.

[4]U-plate 220-24A. Cooke Engineering Co., Alexandria, Virginia.

[5]Becton, Dickinson and Company, Mississauga, Ontario.

[6]#430-1899 Gibco, Grand Island, New York.

[7]#220-6140 Gibco, Grand Island, New York.

[8]Pfizer, Montreal, Quebec.

[9]Vancocin. Eli Lilly and Company Ltd., Toronto, Ontario.

[10]Gentocin. Schering Corporation Ltd., Pointe-Claire, Quebec.

[11]Bacto-Concanavalin A 3351-56-2. Difco Laboratories, Detroit, Michigan.

[12]3040 Micro Test II. Falcon Plastics, Oxnard, California.

[13](methyl-^{3}H) Thymidine, specific activity 19.0 Ci/mmoL. Amersham, Oakville, Ontario.

[14]Flow Laboratories, Rockville, Maryland.

[15]Minivial. New England Nuclear, Boston, Massachusetts.

[16]pBix (2-(5 phenloxazol) benzene) #NEF902. New England Nuclear, Boston, Massachusetts.

[17]25 diphenyloxazole #NEF901. New England Nuclear, Boston, Massachusetts.

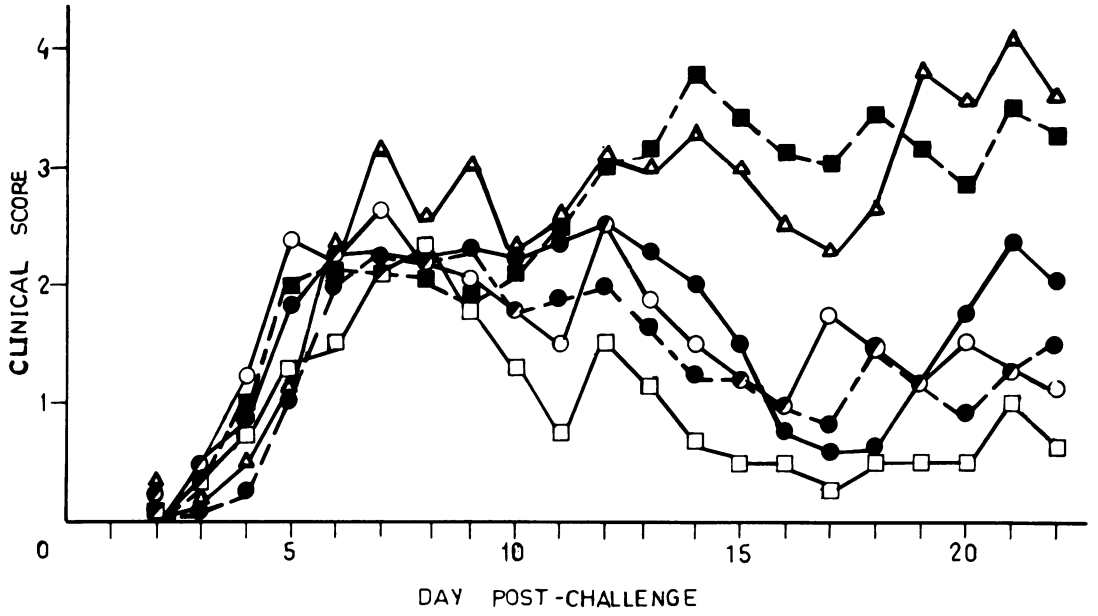

Fig. 1. Mean daily clinical scores in groups of cats after challenge with C. psittaci. Clinical scores defined in Table II. Groups defined by vaccine received prior to challenge as outlined in the key.

Key: △——△ nonvaccinated
□——□ Strain A, live
■----■ Stain A, inactivated
○——○ Strain B, inactivated
●----● Strain B, inactivated, L75 adjuvant
●——● Strain B, inactivated, FD19 adjuvant

in one liter of scintantalized toluene[18]) was added to each vial. These were held overnight in the dark at 4°C. Counts were read in a Searle Delta 300 Beta Counter[19] and recorded as the average count per minute for a three minute period.

RESULTS

CLINICAL SIGNS

All cats appeared healthy at the initiation of the trial and remained well to the time of challenge. None exhibited untoward effects as a result of vaccination with any of the preparations.

Conjunctival swabs cultured prior to challenge were negative for chlamydiae, viruses, mycoplasmas and bacteria. Chlamydiae but no other organisms were isolated from conjunctival swabs from all cats one week after challenge.

Mean daily clinical scores for each group following challenge are plotted in Fig. 1. Conjunctivitis was the predominant sign, with copious serous discharges and in some cats chemosis being the marked features. None of the animals developed purulent conjunctivitis as a result of challenge. In no case did vaccination entirely prevent disease but it would appear that the live vaccine induced the best protection while the inactivated vaccine of the same strain provided virtually none. Statistical analysis confirms this impression (Table III). A significant degree of prophylaxis was also provided by strain B inactivated vaccines, which were equally effective irrespective of the presence of adjuvant.

COMPLEMENT FIXATION TEST

Few animals developed detectable serum antibody, as assayed by the complement fixation test. Those which did respond are listed in Table IV. The ability of a vaccine to stimulate complement fixing antibody was no reflection of its effectiveness in preventing disease. Inactivated Strain B

[18]**Fisher Scientific Company, Fairlawn, New Jersey.**

[19]**G.D. Searle and Company, Arlington Heights, Illinois.**

TABLE III. Comparison[a] of Mean Clinical Scores Between Groups of Cats Vaccinated with Various *C. psittaci* Vaccines and Challenged with 0.5mL 10^4 ELD_{50}/mL Suspension of Virulent Cello Strain *C. psittaci*

Vaccine Groups Compared		Significance level (P)
Unvaccinated to	Strain A, live	0.001
	Strain A, inactivated, L75	no significant difference
	Strain B, inactivated	0.01
	Strain B, inactivated, L75	0.01
	Strain B, inactivated, FD19	0.01
Strain A live to	Strain A, inactivated, L75	0.001
	Strain B, inactivated	0.005
	Strain B, inactivated, L75	0.025
	Strain B, inactivated, FD19	0.025
Strain A inactivated to	Strain B, inactivated	0.01
	Strain B, inactivated, L75	0.01
	Strain B, inactivated, FD 19	0.01
Strain B, inactivated to	Strain B, inactivated, L75	no significant difference
	Strain B, inactivated, FD19	
Strain B, inactivated, L75 to	Strain B, inactivated, FD19	

[a]Comparison made by Student's t test

TABLE IV. Complement Fixation Titers[a] to *C. psittaci* in Cats Vaccinated with Various[b] *C. psittaci* Vaccines

Vaccine	Cat	Pre-V	Pre-C	Post-C
group 1	6 cats, no response			
group 2	ZX5	0	4	4
	01	0	4	4
	4 cats, no response			
group 3	CAL4	0	4	16
	KO	0	2	8
	P1	0	0	8
	3 cats, no response			
group 4	6 cats, no response			
group 5	ZP5	0	0	2
	5 cats, no response			
group 6	CAY2	0	8	8
	CAZ1	0	16	16
	ZX4	0	16	32
	CBC1	0	8	8
	ZV1	0	8	16
	J1	0	16	32

Pre-V = prevaccination Pre-C = prechallenge Post-C = postchallenge
[a]Titer expressed as the reciprocal of the 50% end point
[b]Vaccine groups defined in Table I

with FD19 adjuvant was the only vaccine to induce complement fixing antibodies in all cats (group 6). Even so, signs in this group were comparable to those in the other two groups inoculated with the same chlamydial strain (Table III).

LYMPHOCYTE BLASTOGENESIS TEST

A one-way analysis of variance revealed a significant difference ($P<0.005$) in the stimulation ratios between groups of cats only when strain B antigens were tested 14 days after challenge (Table V). Elevated ratios could be attributed to cats immunized with Strain A live vaccine (group 2). Moreover, within this group an increase in ratios could be demonstrated between prechallenge and postchallenge samples (paired t test, $P<0.05$). This particular group of cats also exhibited the greatest resistance to clinical disease after challenge (Fig. 1, Table III).

DISCUSSION

Experimental challenge with Cello strain *C. psittaci* produced conjunctivitis of vary-

TABLE V. Lymphocyte Blastogenesis Test. Stimulation Ratios (S.R.)[a] for Various *C. psittaci* Suspensions in Cats in Each Vaccine Group

Cat	Antigen	S.R. Pre–C	S.R. Post–C (14)	S.R. Post–C (28)	Cat	Antigen	S.R. Pre–C	S.R. Post–C (14)
group 1: CAO3	A	0.06	0.38	0.21	group 4: CBD1	A	0.19	0.03
	B	0.47	0.28	0.20		B	0.04	0.07
	C	0.07	0.48	0.26		C	0.14	0.03
ZU3	A	0.05	0.07	0.02	CAI3	A	0.02	0.17
	B	0.23	0.04	0.17		B	0.25	0.32
	C	0.19	0.15	0.51		C	1.34	0.09
Q1	A	0.13	0.08	0.01	N1	A	0.10	0.13
	B	0.51	0.08	0.49		B	0	0.09
	C	0.43	0.42	0.63		C	0.14	0.05
group 2: CAA7	A	0.22	0.12	0.12	group 5: ZP5	A	0.07	0.29
	B	0.25	0.85[b]	0.77		B	0.11	0.35
	C	0.02	0.22	0.36		C	0.20	0.27
ZX5	A	0.40	0.68	0.24	CALI	A	0.09	0.11
	B	0.36	0.91[b]	0.30		B	0.19	0.11
	C	0.36	0.30	0.21		C	0.25	0.27
O1	A	0.23	0.59	0.36	L1	A	0.05	0.38
	B	0.39	1.83[b]	0.51		B	0.02	0.42
	C	0.10	0.09	0.57		C	0.19	0.82
group 3: CBB2	A	0.01	0.09		group 6: ZX4	A	0.24	0.03
	B	0.02	0.43			B	0.09	0.05
	C	0.23	0.21			C	0.03	0.01
CAL4	A	0.09	0.14		CBC1	A	0.08	0.02
	B	0.11	0.18			B	0.01	0.03
	C	0.27	0.11			C	0.37	0
P1	A	0.02	0.22		J1	A	0.25	0.01
	B	0.01	0.14			B	0.15	0.02
	C	0.33	0.60			C	0.30	0.03

KEY: Pre–C = before challenge
Post–C (14) = 14 days postchallenge
Post–C (28) = 28 days postchallenge
Antigen: A = strain A, B = strain B, C = challenge strain (Cello)

[a]S.R. = the absolute difference between the $\log_{10}$ of the mean c.p.m. of the test *C. psittaci* suspension and that of a sterile yolk sac suspension

[b]Significant elevation of S.R.

ing severity in all cats. Vaccination with the commercial live preparation induced the best resistance to this challenge. Protection was also good using inactivated preparations of the field strain (B) *C. psittaci*. The superiority of this strain over the inactivated commercial strain (A) cannot easily be accounted for. Both preparations were of similar titer, $10^{7.5}$ and $10^{7.3}$ ELD_{50}/mL respectively. Strain A had, however, been passaged a number of times in embryonated eggs prior to preparation of the commercial vaccine which was used as the source for inactivated organisms and may thus have changed in antigenicity from the original cat isolate (2). Strain B, on the other hand, had been passaged only five times from the initial case of conjunctivitis. Moreover, being a conjunctival pathogen, this strain may be more closely related to the conjunctival isolate used for challenge (7). This would be especially important if, as was found for *C. trachomatis* (27) cross-reactivity between strains of *C. psittaci* is poor.

Assessment of clinical signs appears to be the most reliable method for evaluation of vaccine protection (12, 16). As in other experimental trials (3, 7, 15, 16) determination of complement fixing antibody titers gave no indication of the degree of protection. If, as is the case with the guinea pig (28) and the owl monkey (15), resistance to conjunctival infection in the cat is dependent on a combination of local antibody response and the cell mediated immune response (CMI) the development of circulating antibodies might not be relevant. Unfortunately lacrimal secretions could not be recovered in a volume sufficient for the evaluation of a local antibody response using available techniques. The delayed hypersensitivity skin test which has been used as an *in vivo* correlate of CMI in other species is reported not to occur in the cat (1, 14). However, the use of a lymphocyte blastogenesis test as an *in vitro* assessment of CMI response is promising, although correlation between this test and the *in vivo* response of the cat has not yet been established (9, 25). In this particular case, stimulation could only be recognized in cats which had received live chlamydial vaccine. Moreover, stimulation was not to this strain (A) or the challenge strain but to one to which these animals had no apparent exposure (strain B). Here again, this antigen preparation having undergone relatively fewer yolk sac passages may have retained an antigencity more closely related to challenge organisms after replication in the cat's conjunctival cells.

In summary, vaccination with the live *C. psittaci* vaccine appeared to give the best, although incomplete, resistance to challenge. This protection could not be correlated with the stimulation of complement fixing antibodies but may be related to the induction of a CMI response as assessed by the lymphocyte blastogenesis test. There is some indication that the strain of chlamydia used in vaccination may be important. Therefore, the evaluation of inactivated Cello strain or live Cello or strain B preparations might reveal vaccines of even greater reactivity.

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